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Mucosal microbial parasites/symbionts in health and disease: an integrative overview

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Abstract

Microbial parasites adapted to thrive at mammalian mucosal surfaces have evolved multiple times from phylogenetically distant lineages into various extracellular and intracellular life styles. Their symbiotic relationships can range from commensalism to parasitism and more recently some host-parasites interactions are thought to have evolved into mutualistic associations too. It is increasingly appreciated that this diversity of symbiotic outcomes is the product of a complex network of parasites-microbiota-host interactions. Refinement and broader use of DNA based detection techniques are providing increasing evidence of how common some mucosal microbial parasites are and their host range, with some species being able to swap hosts, including from farm and pet animals to humans. A selection of examples will illustrate the zoonotic potential for a number of microbial parasites and how some species can be either disruptive or beneficial nodes in the complex networks of host-microbe interactions disrupting or maintaining mucosal homeostasis. It will be argued that mucosal microbial parasitic diversity will represent an important resource to help us dissect through comparative studies the role of host-microbe interactions in both human health and disease.

Key words: mucosa, microbiota, bacteria, viruses, innate and adaptive immune responses, parasitic protozoa, parasitic protists, extracellular and intracellular parasites, pathobionts.

Introduction

Intimately associated with the human mucosa functions and defences are the complex microbial communities, called the microbiota, which are increasingly understood to play key roles in myriads of aspects in human health and disease (Belkaid & Hand, 2014; Clemente *et al.*, 2012). Millions of years of refinements have ensured that mammalian mucosa in a state of homeostasis are effective at mediating simultaneously two conflicting and essential functions: (i) facilitate exchanges between the outside and the inside of the body to allow optimal breathing, nutrient and water uptake and reproduction and (ii) mediate protection against physical, chemical and biological insults, with the latter being mainly microbial in nature. Many of the molecules of the mucosal innate defence system are key for mediating interactions with microbes, including receptors sensing microbes and the central components of mucus (mucins and antimicrobial peptides) can be traced back to early phases of metazoans evolution (Bakshani *et al.*, 2018; Schroder & Bosch, 2016). In contrast, some of the effector molecules and cells characteristic of the human adaptive mucosal immune system represent more recent additions to the mucosal armoury against microbes with secretory IgAs (SIgAs), the archetypal antibody in human mucosal secretions, being only shared with reptiles and birds (Smith *et al.*, 2013). The mucosal microbiota form extraordinarily complex microbial ecosystems where bacteria, archaea, microbial eukaryotes and viruses form intricate network of microbe-microbe and host-microbe interactions that can be broadly defined as eubiotic, associated with health, or dysbiotic, associated with disease (Levy *et al.*, 2017; Petersen & Round, 2014)(**Fig. 1**). Eubiotic relationships at mucosal

surfaces are dependent on the functional characteristics of the microbiota community and corresponding finely tuned mucosal innate and adaptive immune responses to microbes, that together are required for harmonious, highly dynamic and continuous host-microbes interactions at mucosal surfaces (Belkaid & Hand, 2014; Clemente *et al.*, 2012; Levy *et al.*, 2017). Finely choreographed host-microbiota interactions are essential to maintain mucosal homeostasis in the broadest possible range of conditions experienced by humans, including variations in diet, exposures to various environmental microbes including pathogens and an increasing range of xenobiotics (Ferreiro *et al.*, 2018; Levy *et al.*, 2017).

Mucosal microbial parasites (also referred to as parasitic protozoa or parasitic protists) are phylogenetically highly diverse and heterogenous that can be broadly distributed across human populations and can contribute to important pathologies but that are also often associated with asymptomatic interactions (Chabe *et al.*, 2017; Lukes *et al.*, 2015). Thus human-microbial parasite symbiotic relationships can range from commensalism to parasitism and more recently some host-parasites interactions are suggested to have evolved into mutualistic associations too (Chabe *et al.*, 2017; Loke & Lim, 2016; Lukes *et al.*, 2015; Stensvold, 2019). Hence these mucosa residents will be referred to here as “microbial eukaryote symbionts” to better capture the diversity of symbiotic interactions mediated by organisms historically typically referred to as parasites (Lukes *et al.*, 2015; Stensvold, 2019). Notably it is increasingly appreciated that this diversity of symbiotic outcomes is the product of parasites-microbiota-host complex network of interactions (Burgess *et al.*, 2017; Clemente *et al.*, 2012; Rowan-Nash *et al.*, 2019),

further highlighting the importance of contextuality for the phenotypic outcome of human-microbe interactions (Belkaid & Hand, 2014; Clemente *et al.*, 2012; Levy *et al.*, 2017). In this editorial a selection of examples will illustrate how mucosal microbial parasites/symbionts (MMPS) can represent disruptive nodes of the host-microbes complex network of interactions underlying mucosal homeostasis and thus contribute to directly or indirectly to mucosal dysbiosis. In contrast, other examples will illustrate the potential of microbial eukaryote symbionts to contribute to eubiosis (Stensvold & van der Giezen, 2018). With these seemingly contradictory considerations in mind, it will be argued that MMPS diversity will represent an important resource to help researchers to dissect the potential causal link between eubiosis and health and dysbiosis and disease through comparative studies. This is a research topic not without controversies and important difficulties and that will require a dramatic increase in the physiological functional characterisation of members of the mucosal microbial communities (Hooks & O'Malley, 2017) including microbial eukaryotes (Chabe *et al.*, 2017; Stensvold & van der Giezen, 2018).

Several papers associated with this Special Issue are derived from talks that were delivered at the EMBO Conference “Anaerobic protists: Integrating parasitology with mucosal microbiota and immunology” (http://meetings.embo.org/event/17-anaerobic-protists)(Dessi *et al.*, 2019; Labruyere *et al.*, 2017; Leitsch, 2017; Miranda-Ozuna *et al.*, 2019; Stensvold, 2019). These are complemented by articles providing broader perspectives on the study of the MMPS (Bartley *et al.*, 2018; Chihi *et al.*, 2019; Deere *et al.*, 2018; Rush *et al.*, 2019; van Gestel *et al.*, 2018; Vargas Rigo *et al.*, 2018). For more in depth coverage of MMPS biology including broader taxonomic

coverage (e.g. Fungi and Helminths), mucosal sites and biology (e.g. lungs, mucus) and topics including parasite genomics, parasite diagnostics and mucosal vaccine, the reader is directed to the following reviews or original papers (Baker *et al.*, 2017; Collins & Belkaid, 2018; Corfield, 2018; Hupalo *et al.*, 2015; Lemieux *et al.*, 2017; Rowan-Nash *et al.*, 2019; Ryan *et al.*, 2017; Serradell *et al.*, 2016).

Mucosal microbial eukaryote diversity, host range and zoonoses

What range of microbial eukaryote symbionts can thrive at our different mucosa, how broadly are they distributed across human populations and what are their host range beyond humans, how genetic diverse are they, what species/genetic lineage are associated with disease and how do these influence the mucosal microbiota and vice versa? These are some of the most basic questions for which we still have relatively limited knowledge for most species. This important knowledge gap currently limits us to properly assess the role in health and disease of microbial eukaryote symbionts and reflects the difficulty of studying mucosal associated organisms and viruses more generally through reductive approaches. A few examples will illustrate the importance of new perspectives one can gain from working on answering these basic questions in humans and animal models. New diagnostic technologies (Ryan *et al.*, 2017) and the increasing number of microbial eukaryote symbionts genome sequence data (Hupalo *et al.*, 2015) are all contributing at providing a better picture of the natural history of MMPS, including non-pathogenic species (Chihi *et al.*, 2019). These in combination with metagenomics surveys (Lokmer *et al.*, 2019) will generate more

comprehensive knowledge on MMPS diversity and host range and their link with health and disease.

The relatively common gut MMPS *Blastocystis* spp., *Dientemoeba fragilis* are reviewed by Stensvold (2019)(both species) and van Gestel *et al.,* (2019)(*D. fragilis*). These species are thought to be common in some populations but there are a number of contradictory datasets in relation to their potential role in both disease and health and issues with the apparent important prevalence variations between populations (van Gestel *et al.*, 2018). Although potentially misleading detection tools can explain some variation between studies (Gough *et al.*, 2019; van Gestel *et al.*, 2018), a combination of environmental and biological explanations are also likely to play a role. An intriguing possibility suggested for *D. fragilis* higher prevalence in some countries is pig farming, which could potentially play a role in its higher prevalence in Denmark and the Netherlands where both humans and pigs cohabit in relatively higher densities (van Gestel *et al.*, 2018). This highlights the importance of considering both human and animal prevalence and study in detail the genetic diversity and phylogeny of the microbial eukaryote symbionts to establish their origins among humans and their potential association with animal reservoirs. This is also relevant for the relatively better known species such as *Giardia*, including in developed countries such as the UK (Horton *et al.*, 2019). A recent survey for *Giardia duodenalis* among cattle in Scotland further illustrates the importance of studying animal populations, where this species was shown to be common across surveyed beef and dairy cattle (~32%) and included genetic lineages associated with human symptomatic infections (Bartley *et al.*, 2018). In another example, vaccination

to protect dogs and cats from *Giardia duodenalis* infections (100% prevalence) in a peri-urban disadvantaged community in Argentina, using an elegant vaccination strategy (Rivero *et al.*, 2010), was shown to reduce dog and cat infections with the concomitant reduction of children infections in the community associated with the vaccinated pets (Serradell *et al.*, 2016). This example illustrates the importance of both the knowledge of the epidemiology of a potential pathogen and the molecular mechanisms underlying surface antigen variation to develop an effective vaccine for relevant hosts to eventually also control infections among humans. Similarly, the prevalence of *Entamoeba* spp., including *Entamoeba histolytica*, among humans, chimpanzees and baboon in the Greater Gombe Ecosystem in Tanzania, where the human and nonhuman primate populations overlap, demonstrated a high level of prevalence (~60% for all *Entamoeba* spp. and ~10% of *E. histolytica*) among all three species highlighting the potential for zoonotic transmission of *Entamoeba* species (Deere *et al.*, 2018). Notably the presence of *E. histolytica* in chimpanzees was apparently never associated with symptoms in the tested population, in contrast to human infections (Deere *et al.*, 2018).

Beyond the gut, an interesting set of data for *Trichomonas vaginalis* and *Trichomonas tenax*, infecting respectively the urogenital tract (Hirt & Sherrard, 2015) and oral cavities (Marty *et al.*, 2017) also highlight the importance of specific and sensitive diagnostics and the knowledge of their distributions beyond humans (Maritz *et al.*, 2014). Through carefully testing the specificity of a molecular diagnostic tool used for *T. vaginalis* it was discovered that some infections of the urogenital tract (three male urine

192 samples) could be due to *T. tenax* rather than *T. vaginalis* (Brosh-Nissimov et
193 al., 2019). A screening across dogs and cats for oral trichomonads also
194 indicated a potential zoonotic source for *T. tenax* from pets (Kellerova &
195 Tachezy, 2017). Genotyping *T. tenax* clinical isolates from humans also
196 established that a subset of genetic lineages are significantly associated with
197 periodontal patients, in addition of being common among the tested
198 population in an affluent setting (35% among patients with periodontitis and
199 19% among healthy controls in the studied French cohort)(Benabdelkader *et*
200 *al.*, 2019). Notably both *T. vaginalis* and *T. tenax* are likely derived from
201 species infecting birds (Maritz *et al.*, 2014) as these two species are
202 respectively more closely related phylogenetically to distinct set of species
203 infecting birds including *Trichomonas gallinae*, common among pigeons, and
204 *Trichomonas gypaetinii* isolated from vultures among other *Trichomonas* spp.
205 isolated from various bird species (Martinez-Diaz *et al.*, 2015). Transfer of *T.*
206 *gallinae* from columbiform to passerines has led to important mortality rates
207 for some passerine species dramatically illustrating the potential for a
208 *Trichomonas* species to jump host and spread rapidly through populations
209 and to become a virulent parasite in some contexts (wild finches such as the
210 common chaffinch) whereas it is often a commensal in others (the
211 columbiform rock pigeon)(Amin *et al.*, 2014). The comparative study of the
212 molecular basis of the interactions between these various *Trichomonas*
213 species and mucosal landmarks required to initiate and sustain the
214 colonisation of various hosts and mucosa will be of great interest and
215 represent a fascinating model system to study MMPS transfers between birds
216 and from birds to mammals, including humans (Maritz *et al.*, 2014).

Symbiosis: from parasitism to commensalism to mutualism

Although a number of MMPS are known to be associated with pathologies, leading to important morbidities and mortality rates in some contexts (Bar *et al.*, 2015; Burgess *et al.*, 2017), many infections by the same species are asymptomatic (Chabe *et al.*, 2017; Lukes *et al.*, 2015; Stensvold, 2019). The outcome of host-microbial eukaryote symbionts interactions are dependent on the combination of the characteristics of the host, the microbial eukaryote and the mucosa microbiota, with increasing evidence for an important role played by cross kingdoms interactions (**Fig. 1**)(Burgess *et al.*, 2017; Rowan-Nash *et al.*, 2019). Inter-kingdom interactions can modulate the inflammatory tone of the mucosa through multiple possible direct and indirect interactions between mucosal microbes, microbes and epithelial cells and microbes and immunocytes (**Fig. 2**). Notably the epithelial cells play key roles in both sensing microbes and orchestrating the mucosal immunological innate and adaptive responses mediated by the combination of epithelial cells and immunocytes **(Fig. 2**)(Levy *et al.*, 2017; Petersen & Round, 2014). Primary immunodeficiencies, due to specific genetic background interfering with epithelial cells and/or immunocytes-microbes interactions, or secondary immunodeficiencies due to infections (e.g. HIV/AIDS) or malnutrition, can dramatically increase the susceptibility of the host to numerous infections including by those of MMPS. This is particularly marked for intracellular parasites such as *Cryptosporidium* and Microsporidia, with the HIV/AIDS pandemic highlighting both the importance of the adaptive immune response in controlling these parasites and the high level of human exposure to these

opportunistic intracellular pathogens from divers zoonotic reservoirs (Khan *et al.*, 2018; Stentiford *et al.*, 2016).

In other contexts, MMPS could provide benefit to their mammalian carrier. Mice carrying the recently described gut trichomonad *Tritrichomonas musculis* were shown to be more resistant to challenges by the bacterial pathogen *Salmonella typhimurium* through enhancing mucosal defences by increasing intestinal inflammation via inflammosome activation and increase of the proinflammatory IL-18 production leading to a T_H1/T_H17 immune response (Chudnovskiy *et al.*, 2016). This higher protection level to *Salmonella* was however associated with a cost as *T. musculis* colonisation was also associated with higher rate of colorectal cancer (Chudnovskiy *et al.*, 2016). This contrasts with helminths infections that tend to inhibit gut inflammation through stimulating T_H2/Treg responses (Cortes *et al.*, 2018).

These contrasting examples illustrate the importance, and potential great value, of increasing our knowledge of the natural history of mammal-MMPS interactions and the importance of studying various microbial eukaryote symbiont species in humans and animal models to dissect the complex host-MMPS-mirobiota interactions to illuminate their influence in both health and disease (Loke & Lim, 2016). Additional examples of potentially beneficial microbial eukaryote symbionts; including *Entamoeba* spp. and *Blastocystis* are discussed in this Special Issue (Stensvold, 2019) and in other contexts (Chabe *et al.*, 2017; Lukes *et al.*, 2015; Stensvold & van der Giezen, 2018) and in the next section.

Microbial eukaryote symbionts/parasite-bacteria-virus interactions

The complex interplay between MMPS, bacteria, archaea and viruses and mammalian host health and disease status is increasingly being uncovered through the study of various microbial cellular species, bacteriophages and eukaryote infecting viruses, different mucosal surfaces and mammalian species, including humans (Burgess *et al.*, 2017; Chabe *et al.*, 2017; Clemente *et al.*, 2012; Rowan-Nash *et al.*, 2019). Here a few examples illustrating the link between these interactions and health and disease are covered with MMPS potentially contributing to either eubiosis or dysbiosis depending on context of the hosts and their associated microbiota and environmental factors such as diet and xenobiotics (e.g. antibiotics) (**Fig. 3**).

Arguably one of the most fascinating and complex example includes *Trichomonas vaginalis* that infect the urogenital tracts (UGT) of humans (Hirt & Sherrard, 2015). A complex set of interactions between *T. vaginalis*, RNA viruses infecting *T. vaginalis* (TVV), the bacteria *Mycoplasma hominis* forming symbiosis with *T. vaginalis* and other bacteria associated with bacterial vaginosis, are all thought to contribute in concert to symptomatic infections, adverse pregnancy outcomes and increase transmission and acquisition of human infecting viruses, including HIV, HPV and HSV-2 (Hirt & Sherrard, 2015; Kissinger, 2015). This is thought to be mediated through several mechanisms including, boosting the inflammatory tone of the UGT, increasing the population of target immunocytes for HIV and induction of microlesions disrupting the mucosal barrier (Kissinger, 2015). Furthermore, although human viruses including HIV and HSV are not known to infect *T. vaginalis*, HIV and HSV viral particles can be internalised by the parasite and potentially be transferred to, and infect, human cells in a new host (Pindak *et al.*, 1989;

Rendon-Maldonado *et al.*, 2003). Although *T. vaginalis* can induce tissue damage and inflammation on its own, TVV and *Mycoplasma hominis* can act synergistically to dramatically boost inflammations associated with *T. vaginalis* infections as reviewed by Dessi and colleagues (Dessi *et al.*, 2019). Dysbiosis associated with infections by *T. vaginalis* is also thought to contribute to the pathobiology of *T. vaginalis* (Fichorova *et al.*, 2017; Mercer & Johnson, 2018). Direct targeting of bacteria peptidoglycans by the parasite through enzymes of bacterial origins (Pinheiro *et al.*, 2018) could potentially contribute to modulate the microbiota bacterial taxonomic composition in addition to contributing to *T. vaginalis* capacity to colonise the mucosal surface. The combination of the parasite and several bacterial species characteristic of dysbiotic vaginal microbiota associated with trichomoniasis, were also recently shown to synergistically affect the integrity of the tight junction complex of the cervicovaginal epithelial cells (Hinderfeld *et al.*, 2019). Notably, treating *T. vaginalis* infections with metronidazole can liberate from the killed parasite TVV particles and/or *Mycoplasma hominis* cells leading to the boosting of inflammation and to infection of human cells by *M. hominis* (Dessi *et al.*, 2019; Thi Trung Thu *et al.*, 2018). These different aspects associated with *T. vaginalis* infections illustrates the intricate associations of the parasite with bacterial (*Mycoplasma*) and viral (TVV) endosymbionts, the bacterial members of the UGT microbiota and how these interactions can influence the parasite pathobiology including increasing human infecting virus transmission rates. These considerations will be important to complement more traditional investigations focusing on the study of specific aspects of host-parasite interactions, such as the potential role of environmental glucose concentration

variation (Miranda-Ozuna *et al.*, 2019) and cell surface and secreted factors such as exosomes (Mercer & Johnson, 2018), in modulating the virulence of the parasite. These examples illustrate dramatically the importance to investigate host-MMPS-microbiota-virus interactions in an integrative manner to develop more refined diagnostics and novel prophylactic and therapeutic strategies to eventually promote reproductive and sexual health more efficiently. It will also be of interest to investigate the possibility that related endosymbionts (to TVV and *Mycoplasma*) are also present in other *Trichomonas* species including bird infecting species and *T. tenax* associated with periodontitis (described in the previous section).

The MMPS *Giardia*, *Entamoeba* and *Cryptosporidium* are also known to be infected by RNA viruses (Gomez-Arreaza *et al.*, 2017). *Cryptosporidium* infected virus are associated with higher rate of the parasite propagation capacity; however, it is not clear if this increases the virulence of such infections. Similarly there is currently no evidence for *Giardia* and *Entamoeba* that their RNA viruses can contribute to boosting the pathobiology of these parasites (Gomez-Arreaza *et al.*, 2017). Complex interplay between *Giardia*, *Entamoeba* and *Cryptosporidium* with bacteria members of the microbiota have also been shown to influence the virulence of these parasites in both negative (e.g. inhibiting infections) or positive ways (e.g. promoting virulence)(Burgess *et al.*, 2017; Rowan-Nash *et al.*, 2019). A remarkable example illustrating the importance of the microbiota in playing a role in reducing the impact of *Cryptosporidium* infection was uncovered when investigating two candidate drugs to treat the parasite. Two novel drugs that had promising properties in initial *in vitro* tests had opposite effect on

342 *Cryptosporidium* infections in a mouse model (Gorla *et al.*, 2014). Although
343 one of the drugs was potent in controlling the parasite, the other drug was
344 shown to actually boost infection levels, which was associated with a
345 significant change in the bacterial taxonomic composition of the gut
346 microbiota, with in particular a dramatic increase of the population of the
347 mucin loving gut bacteria *Akkermansia muciniphila* (2,800-fold increase
348 compared to the pre-treatment state), suggesting a dysbiotic microbiota
349 (Gorla *et al.*, 2014). This was rationalised as an off-target impact of the drug
350 on members of the gut microbiota. Although *A. muciniphila* is considered to be
351 an important mutualist associated with human health (Cani & de Vos, 2017),
352 the significant boost in *Cryptosporidium* infection level could be explained by
353 an excessive degradation by *A. muciniphila* of the mucus protective layer in
354 the gut facilitating access to, and eventual infection of, epithelial cells by
355 *Cryptosporidium*. An apparently similar outcome was observed in a mouse
356 model with a humanised gut microbiota fed with a diet depleted from plant
357 fibbers, which led to the depletion of the mucus protective layers by the
358 microbiota and higher susceptibility to pathogens (Desai *et al.*, 2016). These
359 examples illustrate how environmental factors, including xenobiotics (an
360 antibiotic in the example above) and diet, can influence the mucosal microbial
361 ecology and by doing so modulate the host susceptible to infections by
362 potential pathogens, including MMPS.
363
364 **Antibiotics and vaccines for mucosal parasites/symbionts**
365 In contrast to the availability of a broad range of antibiotic treatment regiments
366 for bacteria, there are far less efficient options to treat with drugs symptomatic

infections due to microbial parasites (Farthing, 2006; Leitsch, 2017). As for bacteria, there is also the issue of microbial parasites developing resistance to existing drugs regiments and for off-target effects on the microbiota (Wypych & Marsland, 2018). Furthermore some patients can develop strong reactions to some drugs including to the commonly used metronidazole targeting anaerobic parasites (Leitsch, 2017). These considerations stimulate continuous research efforts to identify new drugs to treat microbial parasites, either based on modifying existing well established drugs such as 5-nitroimidazole (Leitsch, 2017), or new drugs such as plant derived phenanthrenes (Vargas Rigo *et al.*, 2018). Irrespective of the drug, it is increasingly appreciated necessary to consider their broad impact on the host microbiota, with increasing evidence that antibiotic treatments are being associated with dysbiosis favouring opportunistic pathogens, including pathobionts, and/or leading to a difunctional immune response to microbial and other antigens that can lead to debilitating conditions such as allergies and asthma (Wypych & Marsland, 2018). In the case of the treatment of anaerobic mucosal parasites (such as *Trichomonas* and *Giardia*) with metronidazole/imidazole the anaerobic members of the microbiota will also be affected (Leitsch, 2017). This can contribute to dysbiosis in the gut microbiota in particular where anaerobes are known to play important roles (Wypych & Marsland, 2018)(**Fig. 3**).

In comparison to drug treatments options, vaccines for MMPS are even less well developed. This is due to the combination of the inherent difficulties in developing effective mucosal vaccines (Lycke, 2012) and the complex biology of MMPS, including their capacity to mediate cell surface antigen

variation (Deitsch *et al.*, 2009; Gargantini *et al.*, 2016) and the little knowledge we have on the nature of the host immune response to eradicate MMPS (Chapwanya *et al.*, 2016; Farthing, 2006). One promising strategy that takes advantage of the properties of VSP proteins from *Giardia* (Gargantini *et al.*, 2016) and viral-like particles has great potential to develop novel oral vaccines for various pathogens (Serradell *et al.*, 2019), including a broad range of MMPS in addition to *Giardia* (Serradell *et al.*, 2016).

Conclusion and some speculations

From the examples covered here and in cited publications one can conclude that it might be more appropriate to refer to many extracellular microbial eukaryotic symbionts with various pathogenic potential as pathobionts that is, they are members of the mucosal microbial ecosystems that can become pathogenic in some contexts where host genetic, environment and properties of the microbial community as a whole all play a role (Chow *et al.*, 2011). Acquired immunodeficiencies or transfer of MMPS between different hosts species can lead to sub-optimal interactions with some species becoming pathogenic (Farthing, 2006; Price *et al.*, 2017). In contrast intracellular parasites, including the Apicomplexa *Cryptosporidium* and the Microsporidia, are typically thought to be primarily gut pathogens (Farthing, 2006), as they must directly exploit their host cell energy and metabolites to proceed through their life cycle and in the process compromise the integrity of the epithelial monolayer of the gut (Dean *et al.*, 2016; Farthing, 2006). One aim of this editorial was to illustrate specific aspects of the intricate and complex interactions taking place between MMPS, the other members of the

417 microbiota and their animal or human hosts. These highlight the importance of
418 collaborative research projects integrating parasitology, microbiology,
419 virology, pharmacology and mucosal immunology in the context of both basic
420 and medical and veterinary research on the factors influencing mucosa health
421 and disease. Generating more comprehensive knowledge on the link between
422 these microbial interactions and mucosal and systemic health and disease is
423 undoubtedly one of “the most difficult and challenging scientific endeavour of
424 our time”(Birchenough & Hansson, 2017), as it will need to identify and
425 characterise key aspects of thousands of highly dynamic interactions
426 mediated by a complex cocktail of metabolites, cell-virus and cell-cell
427 interactions involving complex microbial communities, epithelial cells and
428 immunocytes. The knowledge derived from the study of these complex
429 network of interactions will be required to eventually develop much needed
430 novel prophylactic, including mucosal vaccines for overt pathogens, and
431 therapeutic strategies (including highly specific drugs, prebiotics, faecal
432 transplants), to regenerate, maintain and promote human and animal health at
433 mucosal surfaces. It is also suggested that considering microbial eukaryote
434 symbionts/parasites will provide important opportunities for much required
435 comparative studies to delicately dissect key nodes orchestrating mucosal-
436 microbes interactions and how these are causally linked to the specific
437 phenotypic outcomes in their human and animal hosts. Contextualisation of
438 the diversity of both MMPS, the microbiota at large (bacteria, archaea and
439 viruses) and their host within an evolutionary and ecological framework will
440 also likely be important at helping building a more predictive theoretical

framework for the outcome of host-microbes interactions (Amato, 2016; Davenport *et al.*, 2017; Ferreiro *et al.*, 2018; Rook *et al.*, 2017).

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722

723 **Figure legends**

724

725 **Fig. 1.** The now generally accepted new paradigm of microbe-microbe / host-
726 microbe complex network of interactions that can contribute to health
727 (maintaining homeostasis) or disease (inducing excessive inflammation
728 through time and space) status of the animal/human host. The terms eubiosis
729 and dysbiosis relate to the microbiota functional activities associated with
730 respectively health through maintaining mucosal homeostasis (ensuring
731 optimal mucosal functionality) or pathologies due to excess inflammation
732 leading to damage mucosal surfaces and that can also contribute to disrupt
733 systemic physiology and sub-optimal cognitive capacities. See main text and
734 cited references for the conceptual limitations on the use of eubiosis and
735 dysbiosis.

736

737 **Fig. 2.** The complex network of interactions at mucosal surfaces between
738 microbes, epithelial cells and immunocytes modulating the immunological and
739 inflammatory status of the mucosal surfaces. Optimal interactions ensure
740 adequate responses to the presence of members of the microbiota and robust
741 challenges to pathogens and at the same time tolerance to innocuous
742 antigens required to maintain long term functionality of the mucosal surface
743 underlying optimal digestion and nutrient uptake, breathing, or reproduction.
744 Arrows indicate direct (e.g. physical contact) and indirect (e.g. metabolites or
745 signalling molecules) interactions such as infection of epithelial cells by
746 intracellular pathogens (viruses or Microsporidia, both illustrated) and doted
747 arrows indicate indirect (e.g. though metabolites) interactions between

748 illustrated cells. Note in particular the central node/role of epithelial cells that
749 integrate, and in effect coordinate/orchestrate the complex network of
750 interactions between microbes and immunocytes. A virus (several green
751 “stars”) infected epithelial cell is illustrated as is a virus infected trichomonad
752 (one green “star”, see example in the text). In addition, some viruses/phages
753 infect bacteria also contributing to the overall functional properties of the
754 mucosa microbial ecology. Intracellular bacteria (black rectangles) and
755 Microsporidia (blue cell and spores) are also illustrated within epithelial cells.
756 ZO, Zonula occludens - tight junction; ECM, extra cellular matrix. For
757 simplicity the presence of mucus and the glycocalyx interacting with luminal
758 microbes are not shown and only a monolayer of epithelial cells (e.g. as in the
759 intestine) is illustrated.
760
761 **Fig. 3.** Potential role of MMPS in inducing dysbiosis or eubiosis at mucosal
762 surfaces. In the context of dysbiosis this would contribute to the loss of
763 mucosal homeostasis, and by doing so to a number of potential pathologies
764 that eventually will translate in dysfunctional mucosa leading to disease both
765 locally, e.g. mucosa inflammation, or more distal impacts. The illustrated
766 examples include: *Trichomonas vaginalis* (*Tv*) contributing to increasing the
767 vaginal bacterial diversity associated with bacterial vaginosis, a form of pro-
768 inflammatory dysbiosis of the urogenital tract (UGT). *T. vaginalis* infections
769 are also associated with the loss of mutualists in the UGT. *Entamoeba*
770 *histolytica* (*Eh*) can contribute to colitis, mucosa perforation and translocation
771 of both parasites and some member of the gut microbiota into the portal vein
772 and systemic tissues that can contribute to highly damaging systemic and

local inflammations in the digestive tract (DT) and beyond. In contrast, the loss of some microbial eukaryotes, including potentially *Blastocystis hominis* (*Bh*) and some *Entamoeba* spp. (especially, non-*histolytica* species), could contribute to the gut microbiota reduced bacterial diversity associated with a dysbiotic state. Similarly, metronidazole treatments aiming at eradicating anaerobic mucosal microbial parasites such as *Giardia* and *Trichomonas* species, will also contribute at disrupting the mucosal microbiota by killing important bacterial anaerobes and can favour the expansion of bacterial pathobionts in the DT and the respiratory tract (RT). See main text for examples and citations. MMPS can also influence the RT - e.g. (Maritz *et al.*, 2014) - but this is not covered here.

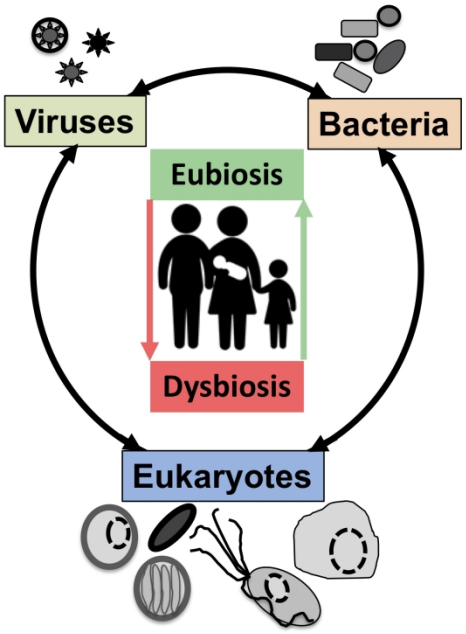

Fig. 1. The now generally accepted new paradigm of microbe-microbe / host-microbe complex network of interactions that can contribute to health (maintaining homeostasis) or disease (inducing excessive inflammation through time and space) status of the animal/human host. The terms eubiosis and dysbiosis relate to the microbiota functional activities associated with respectively health through maintaining mucosal homeostasis (ensuring optimal mucosal functionality) or pathologies due to excess inflammation leading to damage mucosal surfaces and that can also contribute to disrupt systemic physiology and sub-optimal cognitive capacities. See main text and cited references for the conceptual limitations on the use of eubiosis and dysbiosis.

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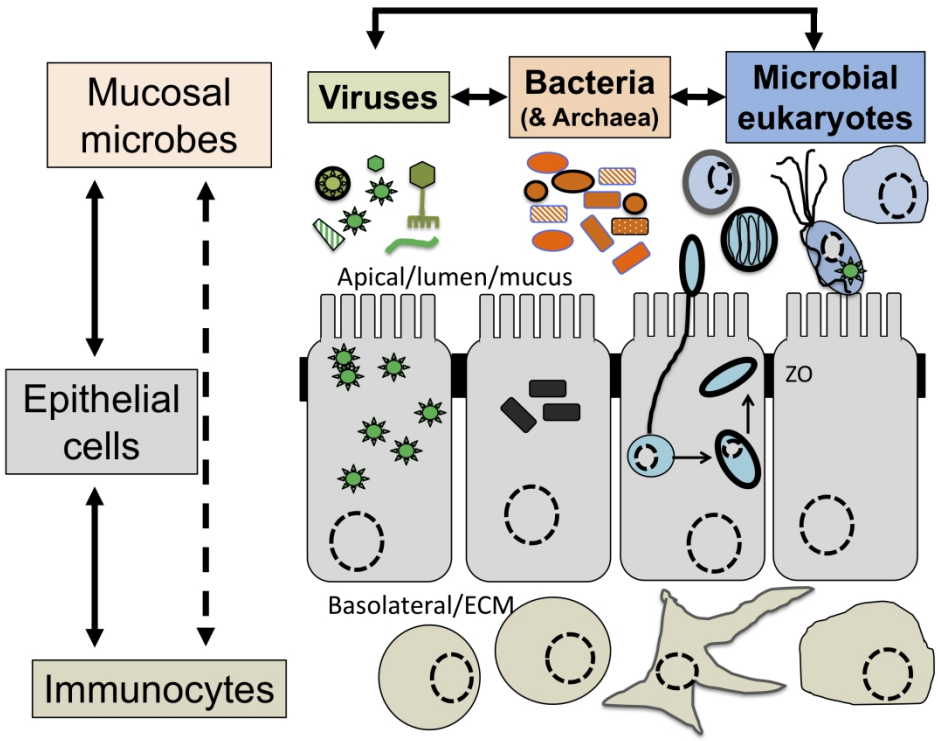

Fig. 2. The complex network of interactions at mucosal surfaces between microbes, epithelial cells and immunocytes modulating the immunological and inflammatory status of the mucosal surfaces. Optimal interactions ensure adequate responses to the presence of members of the microbiota and robust challenges to pathogens and at the same time tolerance to innocuous antigens required to maintain long term functionality of the mucosal surface underlying optimal digestion and nutrient uptake, breathing, or reproduction. Arrows indicate direct (e.g. physical contact) and indirect (e.g. metabolites or signalling molecules) interactions such as infection of epithelial cells by intracellular pathogens (viruses or Microsporidia, both illustrated) and doted arrows indicate indirect (e.g. though metabolites) interactions between illustrated cells. Note in particular the central node/role of epithelial cells that integrate, and in effect coordinate/orchestrate the complex network of interactions between microbes and immunocytes. A virus (several green "stars") infected epithelial cell is illustrated as is a virus infected trichomonad (one green "star", see example in the text). In addition, some viruses/phages infect bacteria also contributing to the overall functional properties of the mucosa microbial ecology. Intracellular bacteria (black rectangles) and Microsporidia (blue cell and spores) are also illustrated within epithelial cells. ZO, Zonula occludens - tight junction; ECM, extra cellular matrix. For simplicity the presence of mucus and the glycocalyx interacting with luminal microbes are not shown and only a monolayer of epithelial cells (e.g. as in the intestine) is illustrated.

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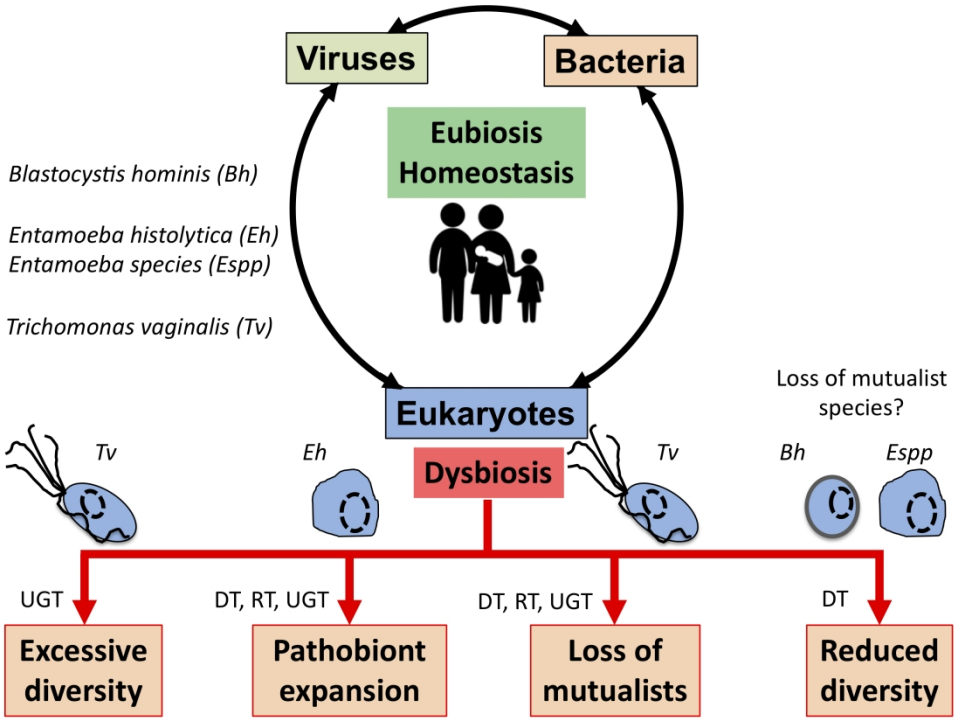

Fig. 3. Potential role of MMPS in inducing dysbiosis or eubiosis at mucosal surfaces. In the context of dysbiosis this would contribute to the loss of mucosal homeostasis, and by doing so to a number of potential pathologies that eventually will translate in dysfunctional mucosa leading to disease both locally, e.g. mucosa inflammation, or more distal impacts. The illustrated examples include: Trichomonas vaginalis (Tv) contributing to increasing the vaginal bacterial diversity associated with bacterial vaginosis, a form of pro-inflammatory dysbiosis of the urogenital tract (UGT). T. vaginalis infections are also associated with the loss of mutualists in the UGT. Entamoeba histolytica (Eh) can contribute to colitis, mucosa perforation and translocation of both parasites and some member of the gut microbiota into the portal vein and systemic tissues that can contribute to highly damaging systemic and local inflammations in the digestive tract (DT) and beyond. In contrast, the loss of some microbial eukaryotes, including potentially Blastocystis hominis (Bh) and some Entamoeba spp. (especially, non-histolytica species), could contribute to the gut microbiota reduced bacterial diversity associated with a dysbiotic state. Similarly, metronidazole treatments aiming at eradicating anaerobic mucosal microbial parasites such as Giardia and Trichomonas species, will also contribute at disrupting the mucosal microbiota by killing important bacterial anaerobes and can favour the expansion of bacterial pathobionts in the DT and the respiratory tract (RT). See main text for examples and citations. MMPS can also influence the RT - e.g. (Maritz et al., 2014) - but this is not covered here.

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